

Supplementary Table S1. Additional baseline characteristics

Characteristics	(N=28)
Smoking status	
Never smoker	13 (46%)
Former smoker	14 (50%)
Current smoker	1 (4%)
Race/Ethnicity	
White	23 (81%)
African American	2 (7%)
Asian	1 (4%)
Declined to answer	1 (4%)
Other	1 (4%)
ECOG PS - no. (%)	
0	14 (50%)
1	14 (50%)

ECOG PS: Eastern Cooperative Oncology Group performance status

Supplemental Table S2: All Treatment Related Adverse Events

All Treatment Related Adverse Event	Total (N=28)	
	All N (%)	Grade 3+ N (%)
Any Events	26 (93%)	6 (21%)
Lipase increased	5 (18%)	4 (14%)
Alanine aminotransferase increased	6 (21%)	2 (7%)
Aspartate aminotransferase increased	6 (21%)	2 (7%)
Blood bilirubin increased	3 (11%)	1 (4%)
Serum amylase increased	8 (29%)	1 (4%)
Hyponatremia	4 (14%)	1 (4%)
Diarrhea	3 (11%)	1 (4%)
Autoimmune disorder	1 (4%)	1 (4%)
Colitis	1 (4%)	1 (4%)
Hepatic failure	1 (4%)	1 (4%)
Rash maculo-papular	8 (29%)	0 (0%)
Pruritus	7 (25%)	0 (0%)
Fatigue	5 (18%)	0 (0%)
Hyperthyroidism	5 (18%)	0 (0%)
Anemia	4 (14%)	0 (0%)
Hyperkalemia	4 (14%)	0 (0%)
Hypomagnesemia	4 (14%)	0 (0%)
INR increased	4 (14%)	0 (0%)
Nausea	4 (14%)	0 (0%)
Alkaline phosphatase increased	3 (11%)	0 (0%)
Constipation	3 (11%)	0 (0%)
Hypoalbuminemia	3 (11%)	0 (0%)
Hypocalcemia	3 (11%)	0 (0%)
Arthralgia	2 (7%)	0 (0%)
Creatinine increased	2 (7%)	0 (0%)
Lymphocyte count decreased	2 (7%)	0 (0%)
Vomiting	2 (7%)	0 (0%)
Anxiety	1 (4%)	0 (0%)
Back pain	1 (4%)	0 (0%)
Cough	1 (4%)	0 (0%)
Dry mouth	1 (4%)	0 (0%)
Dry skin	1 (4%)	0 (0%)
Edema limbs	1 (4%)	0 (0%)
Endocrine disorders - Other, specify-TSH Decrease	1 (4%)	0 (0%)
Endocrine disorders - Other, specify-TSH Elevated decreased	1 (4%)	0 (0%)
Enterocolitis	1 (4%)	0 (0%)
Flatulence	1 (4%)	0 (0%)
Flu like symptoms	1 (4%)	0 (0%)
Gastroesophageal reflux disease	1 (4%)	0 (0%)
Headache	1 (4%)	0 (0%)
Hiccups	1 (4%)	0 (0%)
Hyperglycemia	1 (4%)	0 (0%)
Hypermagnesemia	1 (4%)	0 (0%)
Hypernatremia	1 (4%)	0 (0%)
Hypertension	1 (4%)	0 (0%)
Hypertriglyceridemia	1 (4%)	0 (0%)
Hyperuricemia	1 (4%)	0 (0%)
Hypotension	1 (4%)	0 (0%)
Hypothyroidism	1 (4%)	0 (0%)
Infusion related reaction	1 (4%)	0 (0%)
Investigations - Other, specify-AST increase	1 (4%)	0 (0%)
Investigations - Other, specify-BUN Increase	1 (4%)	0 (0%)
Investigations - Other, specify-Elevated WBC	1 (4%)	0 (0%)

Supplemental Table S2: All Treatment Related Adverse Events

All Treatment Related Adverse Event	Total (N=28)	
	All N (%)	Grade 3+ N (%)
Investigations - Other, specify-Fibrinogen Increase	1 (4%)	0 (0%)
Investigations - Other, specify-Gamma Glutamyl Transferase GGT i	1 (4%)	0 (0%)
Investigations - Other, specify-Increased Fibrinogen	1 (4%)	0 (0%)
Investigations - Other, specify-PTT Increase	1 (4%)	0 (0%)
Investigations - Other, specify-Prothrombin Time Increased	1 (4%)	0 (0%)
Investigations - Other, specify-Total Protein Increase	1 (4%)	0 (0%)
Investigations - Other, specify-fibrinogen increase	1 (4%)	0 (0%)
Lymphocyte count increased	1 (4%)	0 (0%)
Metabolism and nutrition disorders - Other, specify-CO2 Decrease	1 (4%)	0 (0%)
Metabolism and nutrition disorders - Other, specify-Decreased WB	1 (4%)	0 (0%)
Myalgia	1 (4%)	0 (0%)
Nervous system disorders - Other, specify-Left foot pronation	1 (4%)	0 (0%)
Neutrophil count decreased	1 (4%)	0 (0%)
Pain	1 (4%)	0 (0%)
Platelet count decreased	1 (4%)	0 (0%)
Rash acneiform	1 (4%)	0 (0%)
Sinus tachycardia	1 (4%)	0 (0%)
Tinnitus	1 (4%)	0 (0%)

Supplemental Table S3: All Adverse Events Regardless of Relation

All Adverse Event	Total (N=28)	
	All N (%)	Grade 3+ N (%)
Any Event	28 (100%)	15 (54%)
Alanine aminotransferase increased	8 (29%)	4 (14%)
Lipase increased	6 (21%)	4 (14%)
Aspartate aminotransferase increased	7 (25%)	3 (11%)
Anemia	15 (54%)	2 (7%)
Hyponatremia	12 (43%)	2 (7%)
Diarrhea	8 (29%)	2 (7%)
Lymphocyte count decreased	6 (21%)	2 (7%)
Hematuria	5 (18%)	2 (7%)
Urinary tract infection	4 (14%)	2 (7%)
Blood bilirubin increased	3 (11%)	2 (7%)
Serum amylase increased	9 (32%)	1 (4%)
Alkaline phosphatase increased	6 (21%)	1 (4%)
Proteinuria	6 (21%)	1 (4%)
Hypokalemia	2 (7%)	1 (4%)
Arthritis	1 (4%)	1 (4%)
Autoimmune disorder	1 (4%)	1 (4%)
Colitis	1 (4%)	1 (4%)
GGT increased	1 (4%)	1 (4%)
Hepatic failure	1 (4%)	1 (4%)
Hepatobiliary disorders - Other, specify-Hepatitis	1 (4%)	1 (4%)
Infections and infestations - Other, specify-C Diff	1 (4%)	1 (4%)
Metabolism and nutrition disorders - Other, specify-failure to t	1 (4%)	1 (4%)
Surgical and medical procedures - Other, specify-Nephrostomy tub	1 (4%)	1 (4%)
Hypoalbuminemia	9 (32%)	0 (0%)
Rash maculo-papular	9 (32%)	0 (0%)
Fatigue	8 (29%)	0 (0%)
Hyperglycemia	8 (29%)	0 (0%)
Hyperkalemia	8 (29%)	0 (0%)
Hypomagnesemia	8 (29%)	0 (0%)
INR increased	8 (29%)	0 (0%)
Creatinine increased	7 (25%)	0 (0%)
Hypocalcemia	7 (25%)	0 (0%)
Pruritus	7 (25%)	0 (0%)
Constipation	6 (21%)	0 (0%)
Edema limbs	5 (18%)	0 (0%)
Hyperthyroidism	5 (18%)	0 (0%)
Nausea	5 (18%)	0 (0%)
Abdominal pain	4 (14%)	0 (0%)
Activated partial thromboplastin time prolonged	4 (14%)	0 (0%)
Arthralgia	4 (14%)	0 (0%)
Fever	4 (14%)	0 (0%)
Hypertriglyceridemia	4 (14%)	0 (0%)
Cholesterol high	3 (11%)	0 (0%)
Hypernatremia	3 (11%)	0 (0%)
Insomnia	3 (11%)	0 (0%)
Pain	3 (11%)	0 (0%)
Platelet count decreased	3 (11%)	0 (0%)
Vomiting	3 (11%)	0 (0%)
Anorexia	2 (7%)	0 (0%)
Anxiety	2 (7%)	0 (0%)
Back pain	2 (7%)	0 (0%)
Cough	2 (7%)	0 (0%)
Dizziness	2 (7%)	0 (0%)
Dry mouth	2 (7%)	0 (0%)
Dry skin	2 (7%)	0 (0%)
Dyspnea	2 (7%)	0 (0%)

Supplemental Table S3: All Adverse Events Regardless of Relation

All Adverse Event	Total (N=28)	
	All N (%)	Grade 3+ N (%)
Flatulence	2 (7%)	0 (0%)
Headache	2 (7%)	0 (0%)
Hemorrhoidal hemorrhage	2 (7%)	0 (0%)
Hyperuricemia	2 (7%)	0 (0%)
Investigations - Other, specify-fibrinogen increase	2 (7%)	0 (0%)
Lymphocyte count increased	2 (7%)	0 (0%)
Tinnitus	2 (7%)	0 (0%)
Urinary retention	2 (7%)	0 (0%)
Bladder spasm	1 (4%)	0 (0%)
Bloating	1 (4%)	0 (0%)
Blood and lymphatic system disorders - Other, specify-fibrinogen	1 (4%)	0 (0%)
Concentration impairment	1 (4%)	0 (0%)
Dehydration	1 (4%)	0 (0%)
Ear and labyrinth disorders - Other, specify-Right External Ear	1 (4%)	0 (0%)
Endocrine disorders - Other, specify-Increased TSH	1 (4%)	0 (0%)
Endocrine disorders - Other, specify-TSH Decrease	1 (4%)	0 (0%)
Endocrine disorders - Other, specify-TSH Elevated decreased	1 (4%)	0 (0%)
Enterocolitis	1 (4%)	0 (0%)
Flu like symptoms	1 (4%)	0 (0%)
Gastroesophageal reflux disease	1 (4%)	0 (0%)
Gastrointestinal disorders - Other, specify-Belching/ Indigestio	1 (4%)	0 (0%)
Gastrointestinal disorders - Other, specify-tooth sensitivity	1 (4%)	0 (0%)
Generalized muscle weakness	1 (4%)	0 (0%)
Hiccups	1 (4%)	0 (0%)
Hypercalcemia	1 (4%)	0 (0%)
Hypermagnesemia	1 (4%)	0 (0%)
Hypertension	1 (4%)	0 (0%)
Hypotension	1 (4%)	0 (0%)
Hypothyroidism	1 (4%)	0 (0%)
Ileus	1 (4%)	0 (0%)
Infusion related reaction	1 (4%)	0 (0%)
Investigations - Other, specify-AST increase	1 (4%)	0 (0%)
Investigations - Other, specify-BUN Increase	1 (4%)	0 (0%)
Investigations - Other, specify-Beta 2 Micro	1 (4%)	0 (0%)
Investigations - Other, specify-Beta 2 Microglobulin	1 (4%)	0 (0%)
Investigations - Other, specify-CO2 Decrease	1 (4%)	0 (0%)
Investigations - Other, specify-Decreased CO2	1 (4%)	0 (0%)
Investigations - Other, specify-Elevated Chloride	1 (4%)	0 (0%)
Investigations - Other, specify-Elevated WBC	1 (4%)	0 (0%)
Investigations - Other, specify-Fibrinogen Increase	1 (4%)	0 (0%)
Investigations - Other, specify-Gamma Glutamyl Transferase GGT i	1 (4%)	0 (0%)
Investigations - Other, specify-Increased Fibrinogen	1 (4%)	0 (0%)
Investigations - Other, specify-PT Decreased	1 (4%)	0 (0%)
Investigations - Other, specify-PTT Increase	1 (4%)	0 (0%)
Investigations - Other, specify-PTT increased	1 (4%)	0 (0%)
Investigations - Other, specify-Platelet Count Increased	1 (4%)	0 (0%)
Investigations - Other, specify-Prothrombin Time Increased	1 (4%)	0 (0%)
Investigations - Other, specify-Red Blood Cell Decreased	1 (4%)	0 (0%)
Investigations - Other, specify-Total Protein Increase	1 (4%)	0 (0%)
Investigations - Other, specify-WBC elevated	1 (4%)	0 (0%)
Investigations - Other, specify-White Blood Cell Increase	1 (4%)	0 (0%)
Investigations - Other, specify-transferrin decrease	1 (4%)	0 (0%)
Metabolism and nutrition disorders - Other, specify-CO2 Decrease	1 (4%)	0 (0%)
Metabolism and nutrition disorders - Other, specify-Decreased WB	1 (4%)	0 (0%)
Mucosal infection	1 (4%)	0 (0%)
Musculoskeletal and connective tissue disorder - Other, specify	1 (4%)	0 (0%)
Myalgia	1 (4%)	0 (0%)

Supplemental Table S3: All Adverse Events Regardless of Relation

All Adverse Event	Total (N=28)	
	All N (%)	Grade 3+ N (%)
Nasal congestion	1 (4%)	0 (0%)
Nervous system disorders - Other, specify-Left foot pronation	1 (4%)	0 (0%)
Neutrophil count decreased	1 (4%)	0 (0%)
Pain in extremity	1 (4%)	0 (0%)
Rash acneiform	1 (4%)	0 (0%)
Renal and urinary disorders - Other, specify-Difficulty urinatio	1 (4%)	0 (0%)
Renal and urinary disorders - Other, specify-Dysuria	1 (4%)	0 (0%)
Renal and urinary disorders - Other, specify-dysuria	1 (4%)	0 (0%)
Sinus tachycardia	1 (4%)	0 (0%)
Surgical and medical procedures - Other, specify-Ureteroileal Co	1 (4%)	0 (0%)
Thromboembolic event	1 (4%)	0 (0%)
Tremor	1 (4%)	0 (0%)
Urinary frequency	1 (4%)	0 (0%)
Urinary tract pain	1 (4%)	0 (0%)
Urine discoloration	1 (4%)	0 (0%)
White blood cell decreased	1 (4%)	0 (0%)

Supplementary Table S4: Outcomes of patients who did not complete cystectomy as per clinical trial protocol

Patient #	Age and gender	Clinical Stage at Baseline	Reason for no cystectomy	Subsequent treatment	Underwent definitive management	Alive or deceased since Cycle 1, day 1
1	78 F	cT3bN0	Progression in lymph nodes	Chemotherapy (gemcitabine, paclitaxel, adriamycin)	Cystectomy (pCR)	Alive with no evidence of disease at 29.6 months
19	83 F	cT4aN0	Progression with new hydronephrosis	Chemotherapy (gemcitabine, paclitaxel, adriamycin)	Chemoradiation	Alive with no evidence of disease at 19.5 months
28	69 M	cT3bN0	Cystectomy aborted due to gross extravesical disease growing into the pubic rami and pelvic fixation	Chemotherapy (gemcitabine and carboplatin)	Chemoradiation	Alive with no evidence of disease at 14.9 months
33	78 M	cT2N0	Declined by patient; dropped out of study.	Has negative cystoscopy, negative urine cytology and negative CT scan. Patient declined cystectomy and dropped out of study	Declined by patient	Alive with no evidence of disease at 11.8 months

pCR: pathologic complete response

Supplementary Table S5: post-cystectomy complications

Post-surgery, N=24	No. (%)
Median hospital stay, D (range)	6 (3-19)
Median PRBC units, n (range)	0 (0-4)
Median intraoperative blood loss, mL (range)	300 (50-1500)
Diversion type	
Ileal conduit	19 (79)
Orthotopic neobladder	3 (12.5)
Nephroureterectomy	2 (8.5)
Perioperative mortality	0 (0)
Postoperative complications	
Arrhythmia	1 (4)
Ileus	3 (12.5)
Urinary tract infection	1 (4)
Infection with no obvious source	1 (4)
Surgical complications	
Obturator nerve damage	1 (4)

D: day, mL: millilitre

Supplementary Table 6: Key Resource Table

Reagent or Resource	Source	Identifier	Dilution
Antibodies for immunohistochemistry and multiplex immunofluorescence			
Anti-CD20 (L26)	Dako	M075529-2	IHC 1:500 IF 1:250
Anti-CD3 (F7.2.38)	Dako	A0452	IHC 1:100
Anti-CD4 (4B12)	Leica	NCL-L-CD4-368	IHC 1:100
Anti-CD8 (C8/144B)	Thermo Scientific	MS-457-S	IHC 1:100 IF 1:100
Anti-PD-L1 (E1L3N)	Cell Signaling	13684S	IHC 1:100
Anti-ICOS (SP98)	Spring Bioscience	M3980	IF 1:50
Anti-CD4 (EPR6855)	Abcam	AB133616	IHC 1:100 IF 1:50
Anti-FOXP3 (D6O8R)	Cell Signaling	12653	IHC 1:200 IF 1:50
Anti-CD21 (2G9)	Leica	NCL L-CD21-2G9	IHC 1:20 IF 1:10
Chemicals			
Antibody Stabilizer	Candor Biosciences	Cat# 131 050	
Cisplatin	Fluidigm	Cat# 201064	
Cy3	Akoya	Cat# NEL744B001KT	
Cy5	Akoya	Cat# NEL745B001KT	
Cy5.5	Akoya	Cat# NEL766B001KT	
DAPI	Akoya	Cat# FP1490	
EQ 4-element beads	Fluidigm	Cat# 201078	
Opal 480	Akoya	NEL871001KT	
Opal 520	Akoya	NEL871001KT	
Opal 570	Akoya	NEL871001KT	
Opal 620	Akoya	NEL871001KT	
Opal 690	Akoya	NEL871001KT	
Ab Blocking buffer	Akoya	NEL871001KT	
Opal Polymer HRP Ms+Rb 2' Ab	Akoya	NEL871001KT	
BOND Polymer Refine IHC Detection Kit	Leica Biosystems	DS9800	
Ir DNA-Intercalator	Fluidigm	Cat# 201192A	
VECTASHIELD HardSet Antifade Mounting Medium	Vector Laboratories	Cat# H-1400	
Commercial Assays			
X8 Antibody Labeling Kit	Fluidigm	N/A (metal specific)	
Deposited Data			
NanoString data	https://ega-archive.org	EGAS00001004074	
Whole exome sequencing data	https://ega-archive.org	EGAS00001004074	

Software and Databases		
HALO 2.3.2089.70	Indica Labs	Indicalab.com
inForm 2.4.2	Akoya	Akoyabio.com
Vectra Polaris 1.0.11	Akoya	Akoyabio.com
Vectra 3.0.7	Akoya	Akoyabio.com
Bead Normalization	(Finck et al., 2013)	R package premissa
Cytof Workflow	(Nowicka, et al. 2017)	R package cytofworkflow
FlowJo	Tree Star, Inc.	https://www.flowjo.com/
Helios software 6.5.358	Fluidigm	https://www.fluidigm.com/software
Prism 8.0	GraphPad	Graphpad.com
Adobe Illustrator	Adobe	Adobe.com
R 3.6.1	R Core Team (2019)	https://www.r-project.org/
R package: maftools 2.0.16	(Mayakonda et al., 2018)	https://www.r-project.org/
R package: survival 3.2-3	R Core Team (2019)	https://www.r-project.org/
BWA 0.7.5a	(Li & Durbin, 2009)	http://bio-bwa.sourceforge.net/
GATK 3.1-1	(McKenna et al., 2010)	https://gatk.broadinstitute.org/hc/en-us
Picard 1.112	Broad Institute (2018)	https://sourceforge.net
Mutect 1.1.4	(Cibulskis et al., 2013)	http://www.broadinstitute.org/cancer/cga/mutect
Pindel 024t	(Ye et al., 2009)	http://gmt.genome.wustl.edu/packages/pindel/
ANNOVAR 2018-04-16	(Wang et al., 2010)	https://annovar.openbioinformatics.org/en/latest/user-guide/download/
HLA-VBSeq v2	(Wang et al., 2019)	http://nagasakilab.csml.org/hla/
SIFT 6.2.1	(Sim et al., 2012)	https://sift.bii.a-star.edu.sg/
pVAC-Seq 4.0.10	(Hundal et al., 2016); (Hundal et al., 2019)	https://pvac-seq.readthedocs.io/en/latest/
nSolver 4.0	NanoString, Inc.	http://www.nanostring.com/products/nSolver
COSMIC	(Tate et al., 2019)	https://cancer.sanger.ac.uk/cosmic
EXAC	(Lek et al., 2016)	http://biorxiv.org/lookup/doi/10.1101/030338
ESP600	Exome Sequencing Project (ESP), Seattle, WA	https://esp.gs.washington.edu
1000 Genome	The 1000 Genomes Project Consortium, 2015	https://www.internationalgenome.org/
UCSC genome browser	(Kent et al., 2002)	http://genome.ucsc.edu/

Antibodies for CYTOF			
Reagent or Resource	Source	Identifier	Dilution
Anti-CD45 (clone HI30)	Fluidigm	3089003B	1:400
Anti-CD8a (clone RPA-T8)	Biolegend	301053	1:100
Anti-CCR7 (clone G043H7)	Biolegend	353237	1:125
Anti-PD1 (clone EH12.2H7)	Biolegend	329941	1:100
Anti-Eomes (clone WD1928)	eBioscience	14-4877-82	1:167
Anti-CD163 (clone DHI/61)	Fluidigm	3145010B	1:100
Anti-CD11c (clone 3.9)	Fluidigm	3146014B	1:400
Anti-Tbet (clone 4B10)	Biolegend	644825	1:200
Anti-CD16 (clone 3G8)	Fluidigm	3148004B	1:100

Anti-CD66b (clone G10F5)	Biolegend	305102	1:100
Anti-PD-L1 (clone MIH1)	eBioscience	14-5983-82	1:100
Anti-CD123 (clone 6H6)	Fluidigm	3151001B	1:200
Anti-TCRgd (clone 11F2)	Fluidigm	3152008B	1:100
Anti-ICOS (clone ISA-3)	eBioscience	14-9948-82	1:167
Anti-RORgT (clone 600214)	R&D	MAB6109	1:100
Anti-CD45RA (clone H100)	Fluidigm	3155011B	1:200
Anti-CD86 (clone IT2.2)	Fluidigm	3156008B	1:100
Anti-LAG3 (clone 11C3C65)	Biolegend	369302	1:100
Anti-VISTA (clone 730804)	R&D	MAB71261	1:100
Anti-CD14 (clone M5E2)	Fluidigm	3160001B	1:100
Anti-CTLA-4 (clone 14D3)	Fluidigm	3161004B	1:200
Anti-FoxP3 (clone PCH101)	Fluidigm	3162011A	1:200
Anti-BTLA (clone J168-540)	BD	624084	1:100
Anti-CD19 (clone HIB19)	Biolegend	302202	1:167
Anti-TCF7 (clone 7F11A10)	Biolegend	655202	1:125
Anti-TIM3 (clone F38-2E2)	Biolegend	345019	1:200
Anti-GATA3 (clone L50-823)	BD	558686	1:125
Anti-CD73 (clone AD2)	Fluidigm	3168015B	1:500
Anti-CD4 (clone RPA-T4)	Biolegend	300502	1:167
Anti-CD3 (clone UCHT1)	Biolegend	300437	1:100
Anti-CD68 (clone Y1/82A)	Fluidigm	3171011B	1:200
Anti-CD28 (clone CD28.2)	BioLegend	302937	1:125
Anti-GranzymeB (clone GB11)	Fluidigm	3173006B	1:100
Anti-Ki67 (clone Ki67)	Biolegend	350523	1:200
Anti-CD45RO (clone UCHL1)	Biolegend	304239	1:200
Anti-CD56 (clone NCAM16.2)	Fluidigm	3176008B	1:100
Anti-HLADR (clone L243)	Fluidigm	Custom	1:400